

THE EFFECT OF POTASSIUM DEFICIENCY ON THE
REABSORPTION OF PROTEIN IN THE RENAL
TUBULE OF THE RAT*

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Within a few days of feeding rats a diet deficient in potassium characteristic lesions appear in their kidneys (1). These take the form of eosinophilic granules in the cytoplasm of the cells of the collecting tubules. The granules measure from 0.2 to 4.0 microns in diameter and are not seen in the normal collecting tubule. Although many investigations of their nature have been undertaken, it is still uncertain whether they represent material entering the cell from without, or are the result of distortion of a normal intracellular structure. It was first suggested that these granules are damaged mitochondria resulting from the potassium deficiency (2, 3). However, on the basis of electron microscopic studies (1), Spärgo presented evidence that the droplets do not represent altered mitochondria but are the result of other cytoplasmic changes. Although Spärgo did not exclude the possibility that protein or other substances from the tubular fluid might contribute to the make-up of the granules (1), he did not think they were similar morphologically to the hyaline droplets seen in the convoluted tubule cells of the kidney of the rat subjected to "protein overload." On the basis of more recent morphologic examination of the granules with the electron microscope the idea has once again been advanced that the granules represent altered mitochondria (4).

In the experiments described here, the contribution of serum protein reabsorbed from the tubular lumen to the composition of the granules was investigated by labeling the serum protein with T-1824 (Evan's blue dye), which attaches to serum protein when it is injected. This technique, by which serum protein is tagged with dye, has been used by others in the investigation of protein reabsorption mechanisms in the cells of the proximal convoluted tubules

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of the kidney (5, 6). It was found that T-1824-tagged serum proteins were incorporated into the granules of the collecting tubules in the potassium-deficient rat. An increased proteinuria was also found to be present when potassium deficiency had been induced in the rat, whereas reabsorption of protein by the convoluted tubules was diminished in these rats when compared with pair-fed controls.

Materials and Methods

Male Wistar rats, which weighed 200 gm at the start of the experiment, were used. Potassium deficiency was induced by feeding a diet, supplied by the Nutritional Biochemical Corporation of Cleveland, which contained less than 8 mEq of potassium per kg. Control rats were pair-fed a diet similar in all respects to this low potassium diet, except potassium salts were given as a supplement (0.35 gm potassium chloride and 0.5 gm of potassium hydrogen phosphate to each 100 gm of diet).

Three weeks after initiating feeding the experimental and control diets respectively, each rat was injected with 10 mg of T-1824 (Evan's blue dye) in 1 ml of 0.89 per cent saline *via* the tail vein. Equal numbers of rats from both the experimental and control groups were killed the following day, then others from each group were killed 2, 8, and 14 days respectively, after the injection. In the rats from both the experimental and control groups killed on the 1st, 2nd, 8th, and 14th days after injection of T-1824 respectively, the distribution of T-1824 in the kidney was examined. Slices, about 3 mm in thickness, were cut transversely through the middle of the kidney of each rat so as to include the papilla in the plane of section and were fixed in 10 per cent buffered formalin for 24 hours. Frozen sections, 10 microns in thickness, were then made with a sartorius microtome. These sections were examined microscopically to determine the structures stained by the dye.

In a separate experiment a group of five potassium-deficient rats and a group of their pair-fed controls were injected in like manner with T-1824. All the rats were killed 24 hours following the injection, and the T-1824 content of their kidneys was measured by the method of Sellers (5). In brief, this was carried out as follows: After blood had been obtained by aortic puncture in each rat, the kidneys were perfused *in situ* with 500 ml of 0.89 per cent saline at a pressure of 125 cm of saline through an aortic cannula. Kidneys were removed and homogenized in 6 ml of distilled water, and 25 ml of a 1 per cent solution of dioctyl ester of sulfosuccinic acid was added to the resulting suspension. This liberated the T-1824, and the protein was then precipitated by adding 50 ml of cold acetone. The resultant mixture was centrifuged and extracted three times with 50 ml of a mixture of water, and equal amounts of the dioctyl sulfosuccinate solution and acetone. The supernatants were combined, and the T-1824 content was determined by comparison with suitable standards in the spectrophotometer at wave length of 620 $m\mu$.

The T-1824 level in the serum was measured by diluting 0.2 ml of serum with 3.0 ml of 0.89 per cent saline and then comparing the absorption of the resultant solution at 520 $m\mu$ with the absorption of suitable T-1824 standards made up in saline.

Serum protein determinations were carried out by the biuret method of Weichselbaum (7) on the samples obtained by aortic puncture, but a modification of the method was necessary, since T-1824 reacted with the alkaline reagents used in the biuret method and produced a purple color having a different absorption spectrum from that of the T-1824. This purple compound absorbed well at 540 $m\mu$, which is unfortunately the wave length at which the absorption of the copper protein biuret complex is ordinarily measured. To correct for this, the serum containing the T-1824 was allowed to react alone with the alkaline portion of the biuret reagent; the absorption of the resulting color was measured and subtracted from the

absorption found when the reaction was carried out with the full copper-containing biuret reagent. From the resulting absorption value, which represented color due to the reaction of the biuret reagent and the serum protein alone, the value for the serum protein concentration of the sample was determined from a calibration curve of the absorptions of the reaction products of a series of standard protein solutions and the biuret reagent.

In the course of the studies described above, there were daily urine collections on some rats. This was done by placing the animals in individual metabolism cages, suitably arranged to prevent contamination of the collection by food or feces. The urine was collected under toluene, the total urinary volume measured, and the protein content determined on all urine specimens using the method of Shevsky and Stafford (8). The method for protein was checked against Kjeldahl analyses in selected instances.

RESULTS

The Distribution of T-1824 in the Kidneys of the Control and Potassium-Deficient Rats.—The dye was found in the form of droplets in the convoluted tubule cells of the renal cortex of the control animals. These findings are in accord with work by others who have shown that when T-1824 is injected into normal rats, it accumulates in droplet form within the proximal convoluted tubule cells of the kidney. The blue staining of the convoluted tubule cells was most intense in the normal rats killed 2 days after the T-1824 injection. The rats killed 1 day after injection had the next most intense tubular staining, followed by those killed at the 8 and 14 day intervals in that order. In none of the control animals was there any blue dye found in or around the collecting tubule cells.

In all the potassium-deficient rats there was a striking diminution in the amount of T-1824 in the convoluted tubules when compared with appropriate controls. However, the granules which ordinarily occur in the collecting tubule cells as a result of potassium deficiency were lightly stained blue (see Figs. 1 and 2). This was most noticeable in the animals killed 2 days after T-1824 injection. In the animals killed the day after injection, less coloration of the granules was found in the collecting tubules, but a vague blue tint appeared in the collecting tubules as a whole, which suggested that some dye had entered the tubule cells. In the collecting tubules of those potassium-deficient rats injected 2 days previously, those granules which were closest to the lumen were the most deeply stained by the blue dye. The granules present in the interstitial cells of potassium-deficient rats were also stained blue in those rats which had received dye, but this effect was more prominent in the outer part of the medulla than in the papilla.

Urinary Proteinuria in Potassium Deficiency.—During the experiment described above, the wood shavings in the bottoms of the cages were stained blue by the urine of the potassium-deficient rats. While this took place in a moderate degree in the controls, it was never as striking as in the cages of the potassium-deficient rats. Since T-1824 is presumed to be bound to protein in the urine, the increased excretion of T-1824 in the potassium-deficient rats could have

resulted from an increased proteinuria in these animals. Thus measurements of urinary protein were made in a group of four rats, both before and during a period of potassium depletion. For a 2 week control period, the rats were fed the commercial low potassium diet previously described, but adequately supplemented with potassium salts. On the last 2 days of the control period, urine was collected daily on each rat. The potassium supplement was then removed from the diet, and distilled water replaced tap water. A 24 hour urine collection was again made on the 13th, 20th, and 27th day following the initiation of the low potassium diet.

TABLE I
Urinary Protein Excretion Rate of Rats before and after Potassium Deficiency

	During control period*		During potassium deficiency*		
	Day A	Day B	Day 13	Day 20	Day 27
	<i>mg/hr.</i>	<i>mg/hr.</i>	<i>mg/hr.</i>	<i>mg/hr.</i>	<i>mg/hr.</i>
Rat 1	0.29	0.42	1.70	1.00	0.88
Rat 2	0.09	0.22	0.54	0.99	0.45
Rat 3	0.33	0.43	1.13	0.83	0.70
Rat 4	0.59	0.32	1.20	0.83	0.73
Mean.....	0.33	0.35	1.14	0.91	0.69

* All urine specimens analyzed were 24-hour collections.

The urinary protein excretion rate of the four rats before and after the initiation of the potassium deficiency is set out in Table I. It is clear that each animal showed an increased rate of protein excretion while the potassium-deficient diet was fed. It should be observed that, although an increased proteinuria was found in all these animals while being fed low potassium diet, the degree of the proteinuria became less as the animals continued on the diet for a longer period of time.

Renal Tubular Reabsorption of Protein in the Potassium-Deficient Rat.—In view of finding increased proteinuria in the potassium-deficient rats and decreased amounts of T-1824 in the renal cortex of these animals, it was inferred that the normal protein reabsorption process in the proximal tubules was interfered with in these rats. In order to test these findings more precisely we measured the amount of T-1824 taken up by the kidney of the potassium-deficient rat after the dye had been injected intravenously to label the serum protein. The amount of accumulation of T-1824 in the convoluted tubules of the kidneys of the rat under such conditions has been used by others to determine

the rate of tubular reabsorption of protein in the rat kidney. The results of our experiment are given in Table II.

It is apparent that less T-1824 was present in the renal tissue of the potassium deficient rats than in the controls. Also, there was less T-1824 present in the

TABLE II
Renal Tubular Reabsorption in Potassium-Deficient Rats

Body weight	Renal T-1824	Serum T-1824	Serum protein	Renal T-1824 Serum T-1824
<i>gm</i>	<i>mg</i>	<i>mg per cent</i>	<i>gm per cent</i>	
Control rats				
245	0.50	44	6.4	11.36
243	0.80	59	6.05	13.56
247	0.51	52	6.05	9.81
241	0.73	59	6.25	12.37
263	0.64	53	6.54	12.80
Mean..... 247 ± 4*	0.63 ± 0.07	53 ± 2.6	6.25 ± 0.10	11.98 ± 0.65
Potassium-deficient rats				
254	0.26	45	4.96	5.78
280	0.61	59	5.65	10.34
217	0.38	48	5.35	7.92
242	0.17	17	4.51	10.00
233	0.13	36	6.05	3.61
Mean..... 245 ± 11	0.31 ± 0.04	41 ± 3	5.30 ± 0.34	7.53 ± 1.30

The total T-1824 content of both kidneys of the potassium-deficient rats and their controls together with the concentration of T-1824 and protein in the respective sera. In the right hand column the effect of variations in serum T-1824 content on renal T-1824 content is excluded by dividing the renal content by the serum content multiplied by 10^{-3} .

* ± standard error of mean.

serum of the potassium-deficient rats than in the controls. This latter finding might be expected to result were dye-labeled protein to be lost more readily in the urine of the potassium-deficient rat than in the control. Since both the controls and the potassium-deficient rats were given the same amount of T-1824, this substance must have been removed from the serum more rapidly in the potassium-deficient rats than in the controls. It might be argued that in the potassium-deficient rats the low renal T-1824 content was the result of their low serum T-1824, since a low serum level of T-1824 in them would have re-

duced the amount of T-1824 in the glomerular filtrate available for reabsorption by the tubules. Such an interpretation can be examined by comparing the renal T-1824 of the potassium-deficient rats and of the controls with their respective serum T-1824 levels. Such a comparison is shown in the last column of Table II, where the renal T-1824 content of each rat is set out as a ratio of the serum T-1824. Clearly, the renal T-1824 is less in the potassium-deficient rats than in the controls, even when allowance is made for the slightly different serum T-1824 levels in the two groups.

DISCUSSION

It is generally assumed that the T-1824 which selectively accumulates in the renal convoluted tubular cells following its injection into normal rats has been carried there in a serum protein-dye complex (6). The entry of the T-1824 protein complex into the convoluted tubule cells of the normal rat presumably involves mechanisms that ordinarily reabsorb serum protein from the glomerular filtrate. There is much evidence for these assumptions. For instance, when a small amount of T-1824 is injected into rats, as given in these experiments, it quickly becomes bound to the serum protein, and even after a much larger injection of the dye, no measurable free T-1824 is found in the serum after 5 minutes (5). Furthermore, the urinary protein from rats injected with T-1824 is firmly bound to dye, whereas no unbound dye is found in the urine (6). The dye-protein complex is very stable indeed, and since the dye-protein complex of the urine has the same electrophoretic characteristic as that of the serum (9), its appearance in the urine is strong evidence that the dye-protein complex passes the glomerular filter. Oliver has shown that the hyaline droplets, which appear in convoluted tubule cells of rats after the intraperitoneal administration of foreign protein, contain the foreign protein and stain with T-1824 if it be injected simultaneously with the protein (10). It appears probable that in such a situation the dye-protein complex is reabsorbed by the tubular cells in the same manner as the protein alone.

For the same reasons as apply in the interpretation of T-1824 staining of convoluted tubule cells in the normal rat injected with the dye, it is inferred that the coloration of the granules of the collecting tubule cells by T-1824 in the potassium-deficient rat, as demonstrated in this study, represents the result of entry of the dye-protein complex into the collecting tubule cells. It is most likely that the dye-protein complex entered from the lumen, and this idea is strengthened by the finding that those granules closest to the lumen were the ones most heavily colored. A greater intensity of dye in the granules of the rats killed 2 days after dye injection than in those killed 1 day after injection is also consistent with the idea that the dye entered from the tubular lumen, for a

more prompt coloration of granules might be expected to occur were the peritubular capillaries the source of the dye-protein complex.

While T-1824-stained granules were found in the collecting tubules of the potassium-deficient rats, the dye was present in lesser amount in the convoluted tubule cells of such rats than in those of the control rats. This histological observation was confirmed by measurements made of T-1824 renal uptake. Such a decrease in convoluted tubule T-1824 might be expected were less protein filtered at the glomerulus. Instead the potassium-deficient rats showed an increased proteinuria which cannot occur if there be any diminution in the amount of filtered protein, unless at the same time a diminished reabsorption of protein in the tubule takes place. For this reason, the diminished T-1824 accumulation in the convoluted tubules of the potassium-deficient rats probably reflects a decrease in their capacity to reabsorb protein.

It has not yet been ascertained what cellular constituents, if any, contribute to the formation of the droplets in the convoluted tubule cells following protein overload in the rat. While Oliver (11) has presented evidence that the breakdown products of altered mitochondria contribute to the composition of overload droplets, Straus has evidence that the droplets may be allied to those cellular organelles which are called lysosomes (12). Straus injected horseradish peroxidase intravenously into rats and studied the distribution of the peroxidase in the renal tubular cells (12). There was found an accumulation of the peroxidase in granules (12), which Straus called phagosomes, and which had a high concentration of hydrolytic enzymes of the same nature de Duve has discovered in the cellular organelles to which he attached the name lysosomes (13). It is attractive to speculate that the granules in the collecting tubule cells of the potassium-deficient rats may be of lysosomal origin, and thus contain hydrolytic enzymes commonly associated with the lysosome (13). This hypothesis would offer an explanation of the appearance of acid phosphatase in collecting tubule cells and interstitial cells of the papilla of rats shown to take place during the course of potassium deficiency (14), because acid phosphatase is one of the enzymes associated with the lysosome. More precise localization of acid phosphatase and other lysosomal enzymes, such as glucuronidase, to the granules that appear in collecting tubule cells of potassium-deficient rats would lend support to such an hypothesis.

SUMMARY

Male Wistar rats were made potassium-deficient by feeding a diet low in potassium, while controls were pair-fed the same diet supplemented with potassium. Four weeks later 10 mg of T-1824 was injected into each rat. It was found that the characteristic granules which accumulate in the renal collecting

tubule cells as a result of potassium deficiency were colored blue and that a diminished coloration of the convoluted tubule cells of the kidney was present. Quantitative measurements of the renal T-1824 content showed that it was decreased as a result of potassium deficiency. The daily rate of protein excretion was increased by the potassium deficiency. It is concluded that potassium deficiency decreases reabsorption of protein in the convoluted tubules of the kidney and that increased proteinuria thus results. Also, the granules which appear in renal collecting tubule cells as a result of potassium deficiency contain serum protein, which probably enters the cells from the tubular lumen.

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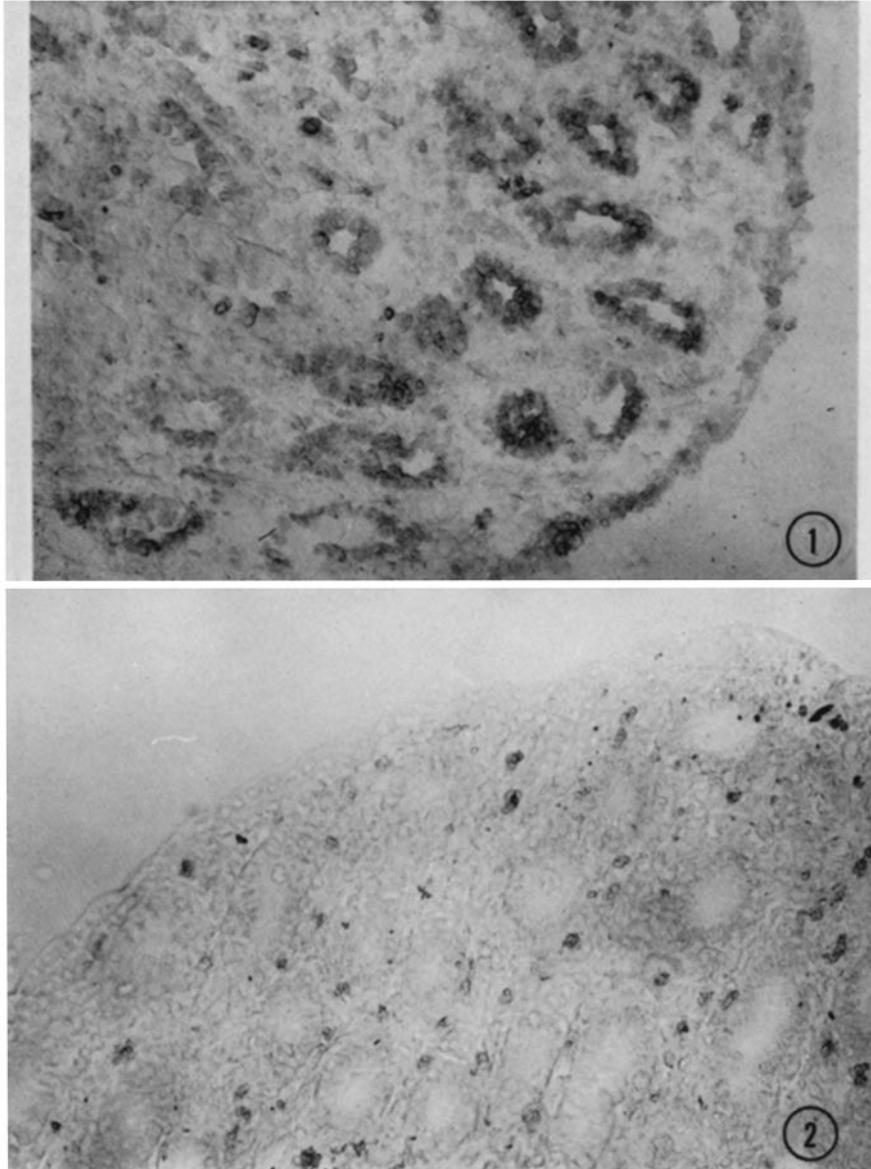
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EXPLANATION OF PLATE 48

FIG. 1. This is a 10 micron frozen section of the renal papilla of a potassium-deficient rat which had been injected with 10 mg of T-1824 intravenously 48 hours before it was killed. The dye is represented by the opacity of the cells of the collecting tubules. The cells of the epithelium covering the papilla are also stained. In the interstitium of the papilla occasional small areas are seen which, on the whole, do not have the intensity of the collecting tubules; these are probably interstitial cells into which the dye has penetrated. $\times 120$.

FIG. 2. This is a 10 micron frozen section of the renal papilla of a potassium-deficient rat. It serves as a control for the section shown in Fig. 1 since no dye was given to the animal. In order to bring out even the minimum of tissue detail it was necessary to "stop down" the condenser of the microscope much more than was the case in Fig. 1. The very slight contrast between the granule-containing collecting tubules and the interstitium has thus been emphasized to its greatest extent. $\times 120$.



(Morrison and Gardner, Jr.: Reabsorption of protein in renal tubule)